# DNA REPLICATION

## SECOND EDITION

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**COVER:**

A looped rolling circle of the duplex replicative form of phage $\phi$X174, based on an electron micrograph provided by Dr Jack Griffith. See Fig. 8-13 for more detail. (Design by Robert Ishi)

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thesis by 5-fluorodeoxyuridylate (FdUMP) requires methylene tetrahydrofolate for covalent binding to thymidylate synthase. Because the inhibition of DHFR by methotrexate prevents regeneration of the tetrahydrofolate, the supply of methylene tetrahydrofolate is depleted and FdUMP is unable to form the ternary complex necessary for prolonged inhibition. With the level of dTTP and its ratio to dUTP diminished, the incorporation of uracil into DNA is increased (Section 15-10) and breakage of DNA is more frequent.

### An Inhibitor of Nucleoside Triphosphate Synthesis

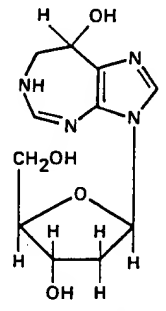
*Cyclamidomycin* (pyracrimycin A, desdanine)[18] is a specific inhibitor of nucleoside diphosphate kinase in *E. coli*. This ubiquitous enzyme produces NTPs, including ATP, and is thus crucial to all macromolecular syntheses.

### Catabolite Analogs

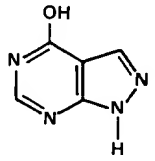
The effectiveness of biosynthetic analogs often depends on their being converted to the nucleotide form and avoiding removal by catabolic enzymes. Potentiation of the antitumor and toxic activity of 6-mercaptopurine by *allopurinol* (Fig. 14-4) may be due to inhibition of xanthine oxidase by the latter (Section 3). Inhibitors of adenosine deaminase not only potentiate the antitumor activity of adenosine analogs, but one of these, *2′-deoxycoformycin* (pentostatin; Fig. 14-4), even when administered alone, produces remissions in patients with acute leukemia derived from T lymphocytes.[19]

## 14-3 Nucleotide Analogs Incorporated into DNA or RNA[20]

Certain analogs of the NTPs, modified in the sugar or base, are accepted by polymerases for pairing with the DNA template and are incorporated into nucleic acid, but subsequently block further chain growth or interfere with nucleic acid functions (Table 14-3 and Fig. 14-5).




2′-Deoxycoformycin




Allopurinol

**Figure 14-4**
Catabolite analogs.

18. Saeki T, Hori M, Umezawa H (1975) *J Antibiot 28*:974.
19. Cass CE (1979) in *Antibiotics V* (Hahn FE, ed.). Springer-Verlag, New York, p. 85.
20. Elion GB (1989) *Science 244*:41.

Table 14-3
**Nucleotide analogs incorporated into DNA or RNA**

| Analog | Incorporated into DNA or RNA | Inhibition |
|---|---|---|
| CHAIN TERMINATORS | | |
| 2′,3′-Dideoxy-NTPs<br>AZT (azidothymidine)<br>Arabinosyl NTPs (araC, araA) | DNA | chain growth, 3′→5′ exonuclease |
| Acyclovir NTP | DNA (analog of G) | chain growth (herpes DNA polymerase) |
| Cordycepin TP (3′-deoxyATP)<br>3′-Amino ATP | DNA, RNA | chain growth |
| DEFECTIVE NUCLEIC ACID | | |
| Uracil dNTP (dUTP) | DNA (analog of T) | DNA integrity: excision leads to chain breakage |
| 5-Hydroxyuridine TP<br>5-Aminouridine TP | RNA | syntheses and functions of DNA and RNA |
| 5-Bromouracil dNTP<br>5-Iodouracil dNTP | DNA (analog of T) | fidelity of replication (mutation); differentiation |
| 5-Azacytidine TP | RNA, DNA (analog of C) | processing of rRNA (defective) |
| Allopurinol (NTP)[a] | RNA (analog of A) | xanthine oxidase; antiprotozoal |
| Tubercidin TP<br>Toyocamycin TP<br>Formycin<br>7-Deazanebularin | DNA, RNA | syntheses and functions of DNA and RNA |
| 2-Aminopurine dNTP<br>2-Aminoadenine dNTP<br>(2,6-diaminopurine) | DNA (analog of A) | fidelity of replication (mutation) |
| 6-Thioguanine dNTP | DNA (analog of G) | fidelity of replication (mutation) |
| UNCLASSIFIED | | |
| 2′-Deoxy-2′-azidocytidine NTP | | initiation of polyoma DNA synthesis; *E. coli* primase |

[a] 4-Hydroxypyrazolo(3,4-d)pyrimidine NTP.

### Chain Termination

The *2′,3′-dideoxyribonucleosides* (Fig. 14-5), if converted to the triphosphates (ddNTPs), are incorporated into DNA at a very slow rate. In studies with *E. coli* pol I, the discrimination does not appear to be in the binding or base pairing of the analog but occurs at a subsequent stage in the reaction when the analog proves inadequate as a primer for the next polymerization event (Section 4-7). Because the analog lacks a 3′-OH group, proofreading excision of the analog is also exceedingly slow, and thus the block of chain growth is maintained. The strikingly specific inhibition of eukaryotic DNA polymerases $\beta$ and $\gamma$ (Section 6-1) suggests that strongly competitive binding by ddNTPs blocks the actions of these enzymes.

*Acyclovir* [9-(2-hydroxy-ethoxymethyl) guanine, acycloguanosine; Fig. 14-5] is one of several synthetic nucleoside analogs [9-(2,3-dihydroxypropyl) adenine is another][21] that are potent inhibitors of herpes simplex viruses. Acyclovir is

21. De Clercq E, Holy A (1979) *J Med Chem* 22:510; King GSD, Sengier L (1981) *J Chem Res (M)*:1501.

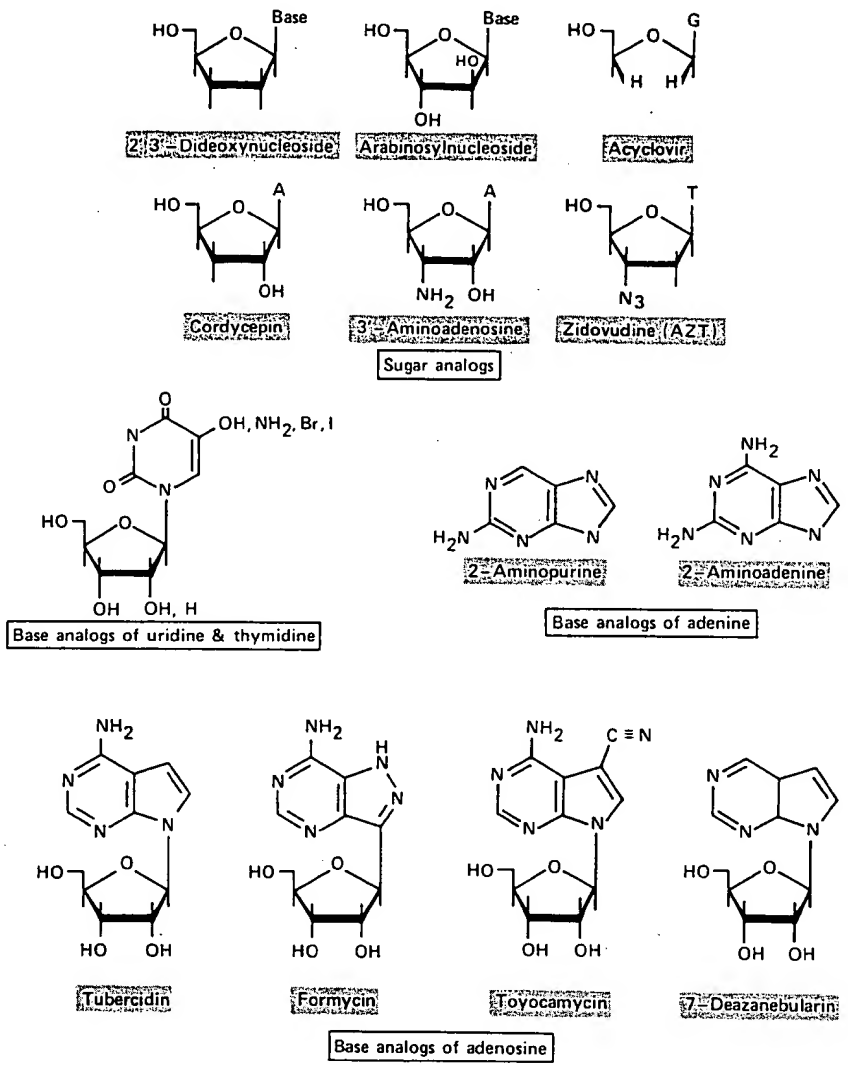



**Figure 14-5**
Nucleotide analogs incorporated into DNA or RNA.

effective as a drug because the analog is phosphorylated to acycloGMP by the herpes-encoded thymidine kinase, but not by the host cell enzyme. AcycloGMP, when further phosphorylated by host cell kinases to acycloGTP, inhibits the herpes-encoded DNA polymerase by a novel mechanism[22] more profoundly than it does the host DNA polymerase. An x-ray diffraction analysis of the acycloguanosine structure[23] attempts to rationalize the remarkable affinities of the nucleoside analog and its phosphorylated derivatives for these otherwise highly specific enzymes.

*Azidothymidine* (3′-deoxy-3′-azidothymidine, AZT, zidovudine; Fig. 14-5) and *2′,3′-dideoxycytidine* (ddC) are among a group of nucleoside analogs[24] that

22. Reardon JE, Spector T (1989) *JBC 264*:7405.
23. Birnbaum GI, Cygler M, Kusmierek JT, Shugar D (1981) *BBRC 103*:968.
24. Mitsuya H, Broder S (1986) *PNAS 83*:1911; Halmos T, Montserret R, Antonakis K (1989) *NAR 17*:7663; Yarchoan R, Broder S (1989) *Pharmacol Ther 40*:329; Huang P, Farquhar D, Plunkett W (1990) *JBC 265*:11914.

are potent inhibitors of human immunodeficiency virus (HIV) replication in cell culture; several have been used in the treatment of the disease AIDS. The nucleosides, converted to the active triphosphate form, inhibit the HIV reverse transcriptase activity, as well as terminating chains once incorporated. Adverse effects of the active forms of AZT and ddC on the host polymerases include the inhibition of both mitochondrial[25] and nuclear replication.

*Arabinosides*[26] (arabinosyl nucleosides; Fig. 14-5) are notable drugs; *cytarabine* (cytosine arabinoside, araC) is used to treat cancer, and *vidarabine* (adenine arabinoside, araA)[27] is an antiviral agent. Among many possibilities and claims for how the drugs act, the most tenable are based on incorporation of the arabinosides into DNA, where they distort the primer-template and block further DNA synthesis by chain termination. Still, the inhibitory action of araC at the stage of chain growth may be marked with certain of the prokaryotic (Section 5-2) and eukaryotic (Section 6-1) polymerases.

Because araC must be in the nucleoside triphosphate form to be active, circumstances that favor conversion of the nucleoside by deoxycytidine kinase enhance its clinical value. Competing with this kinase in some cells and tissues is a potent cytidine deaminase, which destroys the usefulness of araC as a drug Curiously, the related arabinosyl analogs araT and araU occur naturally in sponges.[28]

*Cordycepin triphosphate*[29] (3′-deoxyATP; Fig. 14-5) inhibits chain elongation by RNA and DNA polymerases by generating an inactive primer terminus. The nucleoside does not inhibit bacterial growth, probably because it is phosphorylated very poorly. The triphosphate strongly inhibits RNA synthesis in ascites tumor and HeLa cell lines and affects the addition of polyA segments to the 3′ end of mRNAs. Like 3′-deoxyATP, *3′-amino ATP*[30] inhibits RNA synthesis in isolated nuclei and extracts of ascites tumor cells by terminating elongation after addition to the primer. Although the nucleoside inhibits DNA synthesis in these cells, extracts show no inhibition of DNA synthesis by the triphosphate. Ribonucleotide reduction or RNA-synthesis–dependent DNA synthesis may be the targets of the inhibition in vivo.

### Defective Nucleic Acid

*Uracil* incorporation into DNA by way of dUTP probably occurs to a significant extent under normal circumstances and can be extensive when the ratio of dUTP to dTTP is elevated (Sections 2-9 and 15-10). Uracil in DNA is recognized as foreign and is excised by an N-glycosylase. Mutants defective in the glycosylase can accumulate uracil to levels nearly equimolar with thymine without obvious malfunction of the DNA. Extreme examples are phages in which thymine is completely replaced by uracil or hydroxymethyluracil (Section 17-8).

*Uridine and deoxyuridine analogs with 5-hydroxy or 5-amino substituents* inhibit the synthesis of DNA, RNA, and protein in *E. coli* and interfere, in undetermined ways, with the functions of the RNA and DNA molecules into which they are incorporated.

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25. Chen C-H, Cheng Y-C (1989) *JBC* 264:11934.
26. Matsushita T, Kubitschek HE (1975) *Adv Microb Physiol* 12:247; Cozzarelli NR (1977) *ARB* 46:641.
27. Fridland A (1977) *Biochemistry* 16:5308; Dicioccio RA, Srivastava BIS (1977) *EJB* 79:411.
28. Bergmann W, Feeney RJ (1950) *JACS* 72:2809; Bergmann W, Feeney RJ (1950) *J Org Chem* 16:981.
29. Suhadolnik RJ (1970) *Nucleoside Antibiotics*. Wiley, New York; Roy-Burman P (1970) *Analogues of Nucleic Acid Components*. Springer-Verlag, New York.
30. Langen P (1975) *Antimetabolites of Nucleic Acid Metabolism*. Gordon and Breach, New York.